

Epidemiological and Biological Variability in Clinical Isolates of *Trichomonas vaginalis* among Women in Najaf/ Iraq,

1999 – 2008

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Abstract

In order to monitor changes in the prevalence of *Trichomonas vaginalis* infection, the records of 4992 female patients complaining from vaginal discharge attending the hospitals and the private laboratories in Najaf/ Iraq in the period 1999-2008 were reviewed. Of these 259 (5.188%) were positive. The prevalence of *T. vaginalis* infection has increased significantly over the reviewed years from 3.004% in 1999 to 7.066% in 2008 ($P < 0.01$). There is an intraspecific variation among different isolates obtained from parasite by enzyme electrophoresis, and there are 16 isolates examined fell in eight groups (zymodems) according to the enzymes (Malate dehydrogenase, Malic, Glucose phosphate isomerase, Glucose -6-phosphate dehydrogenase and Phosphoglucosmutase) patterns, and this possibly may suggest that there is more than one strain of *T. vaginalis* in Najaf province.

الوبائية والتغيرات الحيوية لعزلات سريرية لطيفلي المشعرة المهبلية لدى النساء في محافظة النجف / العراق.

جاسم حميد طاهر / الكلية التقنية الصحية والطبية- كوفة

مفتاح البحث: الوبائية والتغيرات الحيوية لطيفلي المشعرة المهبلية

الخلاصة

لغرض مراقبة التغيرات الحاصلة في انتشار اخماج طفيلي المشعرة المهبلية *T. vaginalis*, تم فحص 4992 امرأة من النساء اللواتي تترددن على المستشفيات الحكومية والعيادات الخاصة وكن يعانين من مشاكل صحية وبصورة رئيسه من أفرزات مهبلية في محافظة النجف خلال عشر سنوات وبالذات الفترة الزمنية الممتدة من عام 1999 ولغاية 2008. أظهرت نتائج الفحص أن النسبة الكلية للخمج للسنوات كافة هي 5.88% وان هناك زيادة معتد بها في أخماج الطفيلي خلال سنوات الدراسة فقد تراوحت نسبة الخمج من 3.004% خلال العام 1999 و 7.006% خلال العام 2008 ($P < 0.01$)، إضافة الى ذلك فقد ظهرت اختلافات نوعية بين مختلف عزلات الطفيلي عن طريق تقنية الترحيل الكهربائي حيث تبين أن هناك 16 عزله تميزت الى مجموعتين تبعاً لشدة الخمج وصنفت الى ثمانية مجاميع (Zymodems) طبقاً الى طراز الانظيمات الطفيلية (ماليديهايدر وجينيز والمالك والكلوكوز فوسفيت أيزوميريز والكلوكوز -6- فوسفيت ديهيدر وجينيز والفوسفوكلوكوميوتيز). من خلال هذه النتائج ممكن اقتراح وجود اكثر من سلالة strain لطيفلي المشعرة المهبلية في محافظة النجف وممكن أن تكون لها تأثيرات مرضية متباينة.

Introduction

The flagellated eukaryotic *Trichomonas vaginalis* is responsible for trichomoniasis, the number one, non-viral sexually transmitted disease (STD) of human genitourinary tract (1). Human trichomoniasis is now widely recognized as a prevalent sexually transmitted disease, capable of causing considerable morbidity (2). Approximately 180 million women worldwide may be infected with *T. vaginalis*. Prevalence estimates vary between populations studies, but range from 5-47% in women and 5-29% in men (3). In Iraq, this disease has become more prevalent among women. The prevalent rate has varied from relatively high rate of 19.6 to 18.2% (4)(5) to moderate rate of 9.7 to 10% (6)(7), to as low as 3.9 to 4.8% (8)(9), was reported. Some biochemical and immunological aspects of *T. vaginalis* infection were studied in Iraqi women (5)(7). The aim of this study was to assess the prevalence of *T. vaginalis* infection in women complaining of vaginal discharge attending the hospitals and the private laboratories in Najaf province in the period 1999 – 2008 and to characterise different isolates of the parasites by using electrophoretic patterns for five enzymes of parasite. The specific reason to select these enzymes for the study is that the trichomonads are aerotolerant anaerobes, degrading carbohydrates incompletely to short – chain organic acids principally acetate, lactate and carbon dioxide (10). Pyruvate is product in the cytoplasm by glycolysis. Part of this pyruvate is reduced to lactate by lactic dehydrogenase and excreted, and part of it enters the hydrogenosomes (11). The enzymes which were selected for study were associated with many reactions of glycolysis, the Krebs (citric acid) cycle, the pentose – phosphate shunt, electron transport. Such observations suggest additional potential targets for drug action. The resistant strains show higher glucose uptake, so the measuring the enzymes activity of carbohydrate metabolism was necessary to identify strain pathogenicity. Isoenzyme technique has become essential in understanding the epidemiology of parasitic diseases, which can be caused by parasitic protozoa and is a promising method for the characterization of trichomonads (12). It is a relatively simple and highly reproducible technique and gives markers. To identify subpopulation of *T. vaginalis* and distinguish them from other trichomonads, this method of identification offers the beginning of a biochemical classification to supplement the present morphological classification (13). Furthermore, the suggestion the possibility of the presence more than one strain of *T. vaginalis* in Iraq, because women with trichomoniasis showed marked differences in severity of the disease as well as in their response to chemotherapy (14). The infection of trichomoniasis was studied in Najaf province. The patients mostly infected at their child bearing age (20 – 40) years, and the highest rate of infection 2.8% was recorded in illiterate patients. The chronic infection forms 75% of the cases, and the acute infection was 25% (15).

Materials and Methods

A total of 4992 women with an age range between 22-49 years complaining of vaginal discharge attending (from January 1999 to December 2008) the four Government hospitals (Al-Hakeem, Al-Zahra'a, Al-Manathira and Al-Sajad) and the private laboratories in Najaf province/Iraq were tested for *T. vaginalis*. Vaginal swabs were collected from patients and control group. The swabs were seeded immediately by breaking the lower portion of the swab (16) in the Bijon bottles containing three ml of Diamond's medium (17) and then

incubated aerobically at 35.5 °C or 37°C for 24 hours. The culture was examined every other day for one week before being considered negative(18).The isolates of *T. vaginalis* which were obtained by the previous method were grown in Diamond's medium from which the agar was omitted (17). Organisms were harvested in their late exponential growth phase by centrifugation at 1000 ×g for 30 min at 4°C and washed 3times in phosphate buffered saline. The supernatant was removed and the pellet stored at -70 °C until used (13). When required, frozen pellet was thawed and homogenised by mixing it with an equal volume of solution (0.015% triton X - 100 in 0.9% normal saline), the cells were sonicated at 4 °C. The mixture was centrifuged at 300 ×g for 60 min at 4 °C. The clear supernatant was removed and either used immediately or frozen as rapidly as possible at -70 °C to be used in the next day for electrophoresis (19). Protein content was measured by Folin – Phenol method (20). The LKB multiphore system with LKB 2197 power supply was used, soluble extract from *T. vaginalis* isolates were stained for the following enzymes, phosphoglucomutase PGM (EC.2.7.5.1.); glucose -6- phosphate dehydrogenase G6PD (EC.1.1.1.49); Malic enzyme ME(EC.1.1.1.40); Malate dehydrogenase MDH (EC.1.1.1.37) and glucose-phosphate isomerase GPI (EC.5.3.1.9). The electrophoretic conditions and the staining method used in enzymes visualization were carried out as described by (19).

Results

Epidemiology

In this work, 4992 female patients complaining of vaginal discharge were subjected to epidemiological and biochemical studies. Infection with *T. vaginalis* was detected in 250 (5%) patients by wet mount examination, and 259 patients had positive cultures giving a prevalence rate of 5.188%. The overall prevalence of parasite was 5.1888% as shown in (tab.1 and fig.1), the overall prevalence of parasite has persistently increased from 3% in the year 1999 to 7% in the year 2008 with the increase being statistically significant ($P < 0.01$).

Table1. Overall distribution of trichomoniasis among female patients in Najaf/ Iraq, 1999 – 2008.

Study years	Number of tested specimens	The number of positives %	Age (year)
1999	466	14 (3.004%)	23-43
2000	432	17 (3.935%)	25-48
2001	520	22 (4.230%)	23-49
2002	513	23 (4.483%)	22-46
2003	366	18 (4.918%)	28-47
2004	310	31 (5.081%)	24-45
2005	459	27 (5.882%)	23-48
2006	639	39 (6.103%)	27-49
2007	520	35 (6.730%)	22-44
2008	467	33 (7.066%)	22-42
Total	4992	259 (5.188%)	25-43

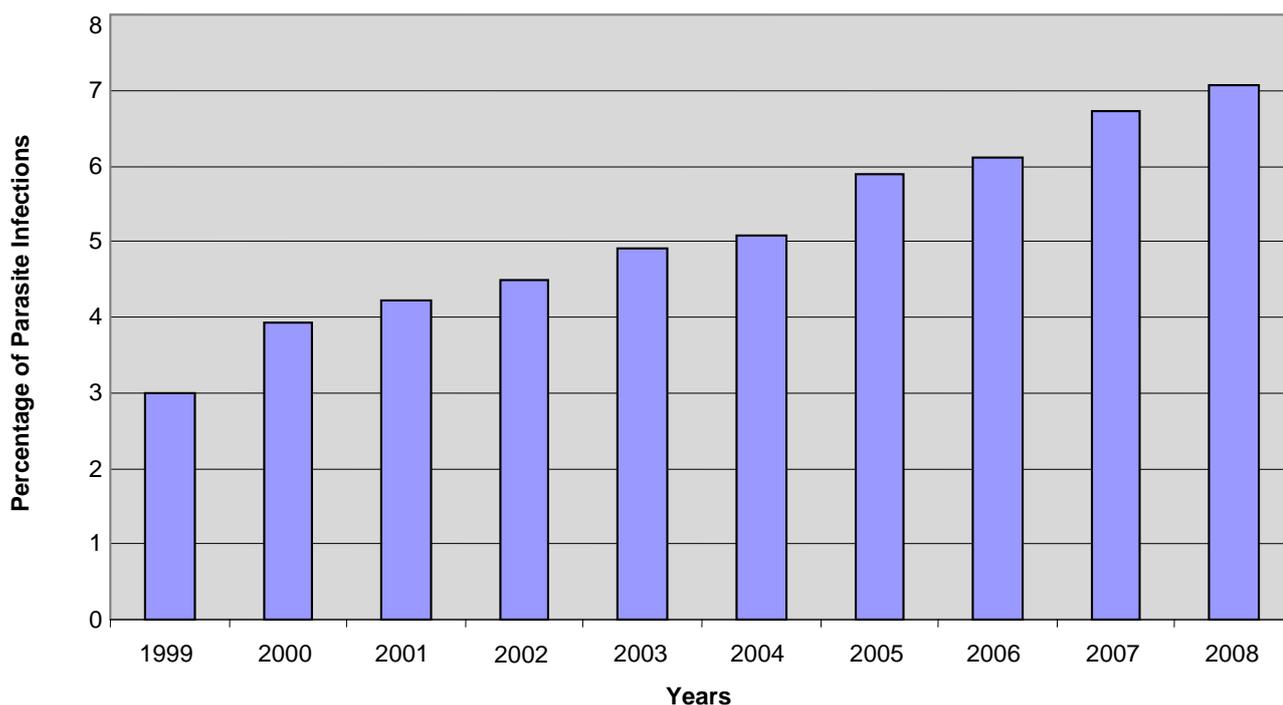


Figure 1 :Distribution of trichomoniasis among female patients in Najaf / Iraq , 1999 – 2008.

Chi square: 391. 72, P<0.01

Enzyme variant types of *T. vaginalis* isolates

The results of electrophoresis of the enzymes examined are summarized in fig. 2. The enzyme patterns appeared within 30 minutes up and to one hour after specific staining. The bands which are variable in their appearance in the electrophoresis were

disregarded. The well defined bands were labeled according to their distance of migration.

- Malate dehydrogenase (MDH)

T. vaginalis isolates showed four different patterns (fig.2), MDH1- pattern with five bands, MDH2- pattern with four bands, the last band near cathode appeared some times as two separate bands when the time of electrophoretic run was slightly prolonged. Only two extracts showed the other two patterns, MDH3- pattern with three bands and MDH4- pattern also with same number of bands.

- Malic enzyme (ME)

Almost all extract of *T. vaginalis* isolates showed the same electrophoretic pattern with three bands. Only nine extracts gave four bands (fig. 2).

- Glucose phosphate isomerase (GPI)

The extracts of *T. vaginalis* isolates showed one broad band of the same molecular weight (fig. 2).

- Glucose -6- Phosphate dehydrogenase (G_6PD)

There are two patterns for this enzyme, one of them consists of one dense bands, the other consists of two bands relatively close to each, but more diffused.

- Phosphoglucomutase (PGM)

Most extracts of *T. vaginalis* isolates of this enzyme showed a single band which was in the same position. Only 13 extracts gave a band slower than the others (fig. 2.).

The enzymatic patterns can be classified into eight groups (zymodemes) according to the severity of the clinical aspects; the 16 isolates studied fell into two groups. The first group represents nine isolates obtained from patients with severe clinical picture (profuse, colour, offensive discharge with itching and soreness) considered as severe infection, while second group includes the remaining seven isolates which were taken from patients with mild to moderate complains small to moderate amount of discharge, with or without irritation called as mild to moderate infection as shown in tab. 2

Table 2. Enzyme variants of 16 *T. vaginalis* isolates obtained by electrophoretic conventional technique.

Bands numbers and arrangement					
Variant code	MDH	ME	GPI	PGM	G ₆ PD
Most virulent isolates					
TH 1.a	2	1	1	2	2
TH 1.b	2	1	1	2	2
TH 1.c	2	1	1	2	2
TH 1.d	2	1	1	2	2
TH 2.a	1	1	1	2	2
TH 2.b	1	1	1	2	2
TH 3.a	2	1	1	1	—
TH 3.b	2	1	1	1	—
TH 4.a	2	2	1	1	—
Intermediate and least virulent					
TH 5.a. 1	2	1	1	2	1
TH 5.a. 2	2	1	1	2	1
TH 5.a. 3	2	1	1	2	1
TH 6.b. 1	1	1	1	1	—
TH 6.b. 2	1	1	1	1	—
TH 7.a	3	1	1	2	2
TH 8.a	4	1	1	2	2

(—) : No active

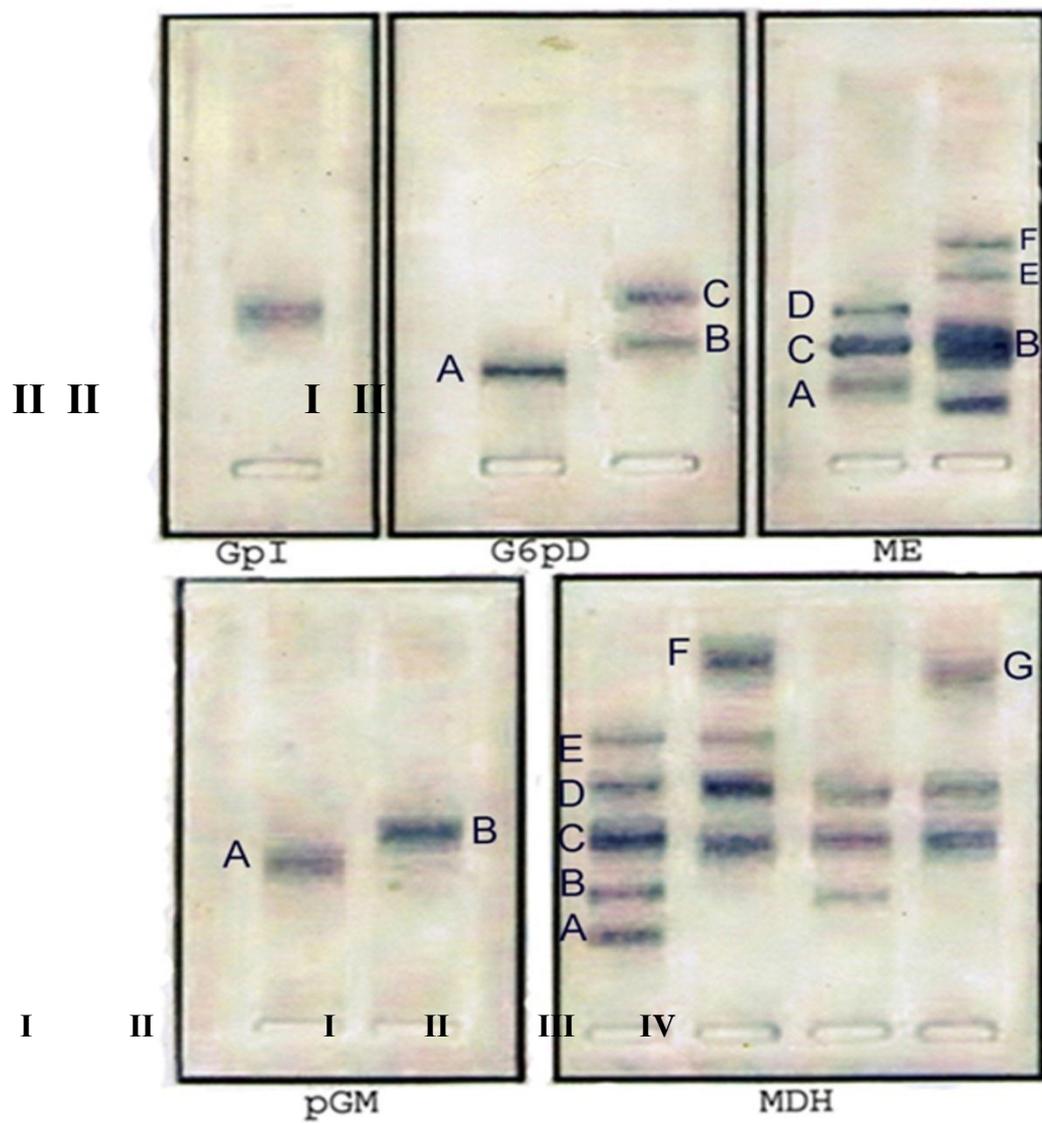


Figure2:Diagrammatic representation of the electrophoretic patterns for *T. vaginalis* enzymes.

Discussion

- Epidemiology

The present work describes the increases observed in the prevalence of *T. vaginalis* infection in a single geographic area along ten years, this is may related to the illegal sex relation and low socioeconomic groups of patients, this may be due to relative lack education and their ignorance in bathroom sanitation and personal hygiene. *T. vaginalis* trophozoites, which are by nature very hardly and resistant to changes in their environment, have been known to survive in urine, on wet sponges, and on damp towels for several hours as well as water for up to 40 minutes. The trophozoites may also migrate through a mother's birth canal and infect the unborn child(21). (22) stated that repeated trichomonal infestation was due to reinfection from untreated partner. (23) reported that a relatively high level resistant noted in strains cultured from patients who did not respond to repeated course of metronidazole, and this lead to what is called persistent for refractory trichomoniasis and the poor compliance with prescribed medication. The important point worth mentioning is the low socio-economic and cultural levels of many emigrate to Najaf province from different localities in Iraq.

- Enzyme variant types of *T. vaginalis* isolated

T. vaginalis strains appear to be different antigenically as assessed by various immunological approaches, but relevance of these differences to parasite virulence and disease is unknown (24). Complicating matters is the paucity of information regarding specific trichomonal membrane components (25), which may be responsible for any heterogeneity or which may be involved in disease pathogenesis (26). So that the identity and function of *T. vaginalis* surface protein antigen that regulate trichomonal heterogeneity were determined by sodium dodecyl sulfate polyacrylamid gel electrophoresis with transfer to nitrocellulose and by immunoblot probed with human sera. All *T. vaginalis* isolates showed similar banding patterns by coomassie brilliant blue and silverstaining of the electrophoresis gels and by amidoblackstaining of the nitrocellulose. However, by immunoblot technique, differences in banding patterns were noted, particularly in high – molecular weight zone (27). The differences between *T. vaginalis* isolates have been defined by different immunological, serological and pathological studies, but, very few data are available on the intraspecific variation of trichomonads using biochemical methods and its finding in relation to other biological properties. The present study shows that it is possible to *T. vaginalis* organisms on the basis of variation in their isoenzyme patterns. All *T. vaginalis* organisms on the basis of variation in their isoenzyme patterns of GPI enzymes should not be regarded as this disadvantage; rather it tends to give confidence in the validity of the species. From the results obtained, some isolates failed to show any bands at all in G6PD enzyme. This could be due to lack of enzyme activity, unsatisfactory conditions for the development, or because in the isolates tested the enzyme is usually labile. The study shows that 16 isolates examined fell in eight groups (zymodems) according to the enzyme patterns of the five enzymes studied. When these results are correlated with the clinical presentation of trichomonal infection in the patients from whom these isolates

were collected, these eight zymodemes were divided into two main groups: severe infection and mild – moderate one. In each group there are four different zymodemes. This reveals that there is an intraspecific variation among the isolates in each group, this may possibly suggests that there is more than one strains of *T. vaginalis* in Najaf province. This is in agreement with finding by many other researchers. (28) suggested that the human genitourinary trichomonads are of different types and possibly even different subspecies, which depend on the pathology and clinical course of trichomoniasis. (13) found considerable differences amongst 32 strains of *T. vaginalis* examined. (29) divided 11 strains into three groups based on four enzymes. (7) suggested that the differences the results of parameters among patients might be due to the fact that there is more than one strain in Iraq. (30) compared three *T. vaginalis* isolates according to their isoenzyme electrophoretic patterns and the metronidazole susceptibility were determined (31). Restriction fragment length polymorphism (RFLPs) and polymerase chain reaction (PCR) may provide a better means of differentiation strains of *T. vaginalis* from patients with varying clinical pictures or from different geographic areas. All the statistical analysis was carried according to (32).

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